EFFECT OF CHRONIC PROGESTERONE OVERDOSAGE ON THE FEMALE ACCESSORY SEX ORGANS OF NORMAL, OVARIECTOMIZED AND HYPOPHYSECTOMIZED RATS

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THREE PLATES (EIGHTEEN FIGURES)

Recent experiments (Selye, '40 a) showed that, contrary to the views expressed in previous publications, the hormone of the corpus luteum when given in the form of large doses of crystalline synthetic progesterone stimulates the growth of the female accessory sex organs without estrin treatment or pretreatment. This effect is demonstrable even in animals in which the simultaneous action of endogenous estrogens is eliminated by ovariectomy. Although it was of some interest to note that none of the effects of progesterone are dependent upon the synergistic action of estrin, the most interesting outcome of these investigations was the demonstration that progesterone is capable of producing full mammary gland development if it is administered in sufficiently large doses and during a sufficiently long period. The factors responsible for the normal development of the breast during pregnancy have puzzled physiologists for a long time because of the intricate mechanism which regulates the growth of this gland. It is well known that during pregnancy, the mammary parenchyma is well developed and much was said about the possible influence which the embryo or the placenta might exert in causing mammary gland growth, until it was seen that a similar development occurs in pseudo-pregnancy in the absence of either (Ancel and Bouin, '11). The fact that nervous

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stimuli play an important part in the development of the gland was made clear by the fact that the stimulus of suckling may lead to full development of the breast with lactation (Selye and McKeown, '34 a, b). In all these instances mammary gland development is accompanied by the formation of functional corpora lutea and progestational changes in the uterus and vagina. It was natural to assume therefore that a secretion of the ovary and probably of the corpus luteum is responsible for mammary gland development especially since ovariectomy prevented the mammotropic effect of mating-, or suckling-pseudo-pregnancy. In pregnant rodents, it was found however that the mammary gland continues to develop in the absence of the ovaries and embryos as long as the placenta is intact. Removal or abortion of the placenta is followed by involution of the breast tissue in such rats. From these and other observations we (Selye et al., '35 a) concluded that the placenta produces corpus luteum hormone. This was confirmed by Takewaki ('38). Since hypophysectomy causes immediate involution of the breast tissue in lactating rats, while ovariectomy has no such effect, it became clear that the maintenance of mammary parenchyma is, in the final analysis, under pituitary control and that the hypophysis, under the influence of hormonal (corpus luteum) or nervous (suckling) stimuli (Selye et al., '34), secretes the principle or principles necessary for breast development. This interpretation is also supported by the demonstration that various so-called "mammogenic" pituitary preparations may cause mammary development even in spayed females or males (Gomez and Turner, '38; Reece and Leonard, '39; etc.). Acceptance of this theory may facilitate the understanding of the otherwise puzzling fact that such a large variety of steroid hormones—namely estrogens, androgens, adrenal cortical steroids, and progesterone—are capable of stimulating the development of the breasts. None of these substances are dependent upon the presence of the gonads for this effect but those so far investigated from this point of view proved to be ineffective in the absence of the pituitary (Selye et al., '35 b,

'36; McEuen et al., '37; Noble, '39). It was logical to assume therefore that all these compounds act merely because they have in common the ability to stimulate the mammogenic hormone production of the hypophysis (Selye and Collip, '36). The only observation which seems to be in contradiction to this theory is that hypophysectomy performed during pregnancy does not interfere with the usual gestational development of the mammary gland nor with the initiation of secretion just before delivery (Selye et al., '33). However this contradiction is only an apparent one if we accept the hypothesis, expressed by Selye et al. ('35 a) according to which the placenta may take over some of the functions of the hypophysis such as its trophic action on the corpora lutea of gestation and on breast tissue.

Since among all the steroid substances so far examined by the author and his co-workers, progesterone proved most active in stimulating the growth of breast tissue, it appeared of interest to undertake a systematic investigation of the mechanism by which this compound exerts its action on the female accessory sex organs.

Our first experiment was merely designed to confirm the effectiveness of progesterone in ovariectomized adult females and to procure material for a detailed histological study of the organ changes which it produces. Twelve female albino rats weighing 126 gm. on the average (range: 101–137 gm.) were ovariectomized for this experiment. Beginning 1 week after the operation six of them were given daily injections of 15 mg. of progesterone daily subcutaneously in 0.5 cc. of peanut oil, the other six acting as untreated controls. All animals were sacrificed on the eleventh day after initiation of these injections. At this time, mere macroscopical inspection of the mammary glands sufficed to show definite acinus formation which revealed itself in the form of numerous fine white granules along the mammary ducts in the progesterone-treated group. Histological sections showed these granules to consist of epithelial cells arranged to form little acini. The structure of the glands was surprisingly compact however in compari-

son with the appearance during the later stages of pregnancy or pseudo-pregnancy and following testosterone or estrin treatment. In the latter cases, there always is at least a trace of milk secretion which distends the acini and thus gives the gland a more follicular structure. No acinus development was seen among the controls whose mammary glands consisted only of fat tissue with a few atrophic ducts (figs. 1 and 2).

The uterus was extremely thin in the controls and showed the usual histological signs of atrophy of the endometrium and myometrium (fig. 3). In sharp contrast to this the progesterone-treated animals all had well developed uteri and histological investigation showed that the endometrium assumed the complicated appearance characteristic of the later stages of pregnancy. It will be recalled that the endometrium of the rat never shows the typical "lace-like" appearance which is so characteristic of progestational proliferation in the rabbit. However in the rat, one may distinguish between two distinct types of progestational proliferation (Selye et al., '35 a). The so-called first progestational reaction is seen very early in pregnancy or pseudo-pregnancy and during lactation. It consists of a relatively smooth surface epithelium with a well-developed compact decidua. This is usually not accompanied by any marked development of the myometrium and the lumen is narrow. Uteri of this type respond easily with deciduoma formation after trauma. The so-called second progestational proliferation which is normally observed only during the second half of gestation is characterized by considerable enlargement of the lumen, excessive development of the muscular coat and a very complicated endometrium whose surface is greatly increased by the formation of numerous folds and crypts. This type rarely responds with deciduoma formation after trauma (Selye and McKeown, '35). In the present experimental series, the uterus usually showed the second type of progestational proliferation (fig. 4). This may explain why we only rarely obtained deciduomata in experiments in which rats were treated with such massive doses of progesterone and then subjected to uterine trauma.

The vagina showed the usual castration atrophy in the controls (fig. 5) while in the progesterone-treated group it began to reveal typical mucification accompanied by the formation of small intercellular cysts filled with eosinophilic colloid (fig. 6). These cysts had previously been noted following progesterone treatment in the vaginal epithelium of intact adult females (Selye et al., '36) and later their appearance was confirmed by Korenchevsky and Hall ('37) in spayed females receiving a combination of estrin and progesterone. In these rats which were treated for 10 days only, neither the mucification nor the cyst formation was as pronounced as in the more chronic experiments to be reported later in this paper. Yet the very fact that such changes occur shows that although simultaneous treatment with estrogens may increase this effect, activation by combined treatment with the two hormones is dispensable. This was not definitely proved by our previous observations (Selye et al., '36) in which mucification and cyst formation were elicited by the administration of progesterone alone in intact females, since in these the possibility of endogenous estrin production had not been eliminated.

The preputial glands also appeared to be stimulated by progesterone since in the castrate controls they weighed only 41 mg. on the average (range: 38–49 mg.), while in the progesterone-treated group they averaged 73.5 (range: 62–97 gm.). The oviducts could not be examined in this series since we removed them with the gonads in order to insure that no trace of ovary was left behind.

The demonstration that all these effects of corpus luteum hormone are obtainable with pure progesterone in the absence of any hypothetical other active principle of the corpus luteum or estrin may clarify some of the contradictory observations reported by previous investigators who used the castrate female rat as a test object. Thus Brouha and Simonnet ('29) claimed that aqueous corpus luteum extracts which inhibit estrus in intact females have no effect on the uterus and vagina of castrates. If, however, they are administered immediately following ovariectomy during estrus, vaginal muci-

fication ensues. This effect has been used by Fevold et al. ('32) as a basis for the assay of the "mucifying hormone" of the corpus luteum. Turner and Schultze ('31) obtained no mammary gland stimulation in spayed females treated with sow corpus luteum extracts. Astwood et al. ('37) and Astwood and Geschickter ('38) who used crystalline progesterone in doses up to 6 mg. per day likewise failed to note any effect on the mammary glands of spayed rats. Korenchevsky and Hall ('37) claimed that spayed females receiving 500 γ of progesterone daily show a very slight increase in the height of the uterine epithelium in comparison with untreated spayed controls but otherwise the uterine structure remained uninfluenced by such treatment. The vagina of these animals likewise remained atrophic although some of the epithelial cells were occasionally transformed into mucous cysts. The average weight of both vagina and uterus were only a trace above that of untreated spayed controls. Kaufmann and Steinkamm ('38) administered 27–309 mg. of progesterone to spayed rats within 61–363 days without noticing any change in the uterus or vagina in comparison with untreated spayed controls. Freud ('39) injected progesterone in doses of 3–9 mg. daily to spayed females during 10 days without noticing any significant action on the vagina unless estrin was simultaneously given. We feel that the present experimental series clearly indicates that there is no reason to postulate the existence of a special "mucifying hormone" different from progesterone and that the latter principle is capable of exerting its influence on uterus, vagina, mammary gland and the female preputial glands in the absence of the ovary and without simultaneous treatment or pretreatment with estrin.

In our second experimental series, twenty-four female albino rats averaging 80 gm. in body weight (range: 75–89 gm.) were divided into four groups. Six served as normal controls and were merely injected with 0.4 cc. of oil; six intact animals were given 10 mg. of progesterone in 0.4 cc. of oil; six were hypophysectomized and given 0.4 cc. of oil and the remaining six were hypophysectomized and given 10 mg. of

progesterone in 0.4 cc. of oil. All injections were administered once daily subcutaneously and the oil used was pure peanut oil. The first injection was given on the day following the ablation of the pituitary and the animals were sacrificed on the eleventh day after initiation of treatment. A detailed discussion of the results is hardly necessary since the vaginal, uterine and preputial gland changes in the normal and the hypophysectomized progesterone-treated animals of this series were the same as those described above as characteristic of the progesterone-treated spayed females. The presence of the pituitary thus proved dispensable for these effects of progesterone. On the other hand, although the mammary glands showed considerable development in the intact progesterone-treated females—a development which was approximately the same as in the previous series of spayed females—no such development was observed in the hypophysectomized animals in which the glands consisted only of atrophic ducts and fat tissue. These results suggested that unlike the other female accessory sex organs, the mammogenic action of progesterone is indirect and mediated by the hypophysis. Before drawing such an important conclusion, it was felt necessary however to ascertain that our inability to influence the mammary glands of the hypophysectomized females was not merely due to a delayed absorption of the injected material which might have been caused by the lowering of the metabolic rate. It appeared conceivable that in the case of such delayed absorption the more sensitive accessory sex organs would have responded while the mammary glands might have failed to be influenced merely because their threshold of response is higher. A repetition of these experiments appeared all the more desirable since they are in contradiction to the findings of Noble ('39) who using smaller doses of progesterone came to the conclusion that the vaginal and uterine actions of this compound are likewise prevented by hypophysectomy.

In order to test the validity of this objection, a third series of experiments was performed on twenty-four female rats under the same conditions as obtained in the previous series.

However in this experiment, the average weight of the animals was 106 gm. (range: 98–116 gm.) and the injections were continued for 20 days, the animals being killed on the twenty-first day. Since the daily dose of progesterone administered during this period was again 10 mg. every treated rat received a total of 200 mg. of this compound during the experiment. It was felt that this enormous dose should certainly suffice to produce mammary gland development in hypophysectomized rats if progesterone has any direct mammogenic effect. It was found however, that while the breasts of the intact animals were fully developed (fig. 7) those of the hypophysectomized rats again consisted only of atrophic ducts and fat tissue (fig. 8). There was but a single exception to this inasmuch as in one rat the breasts were quite well developed, but this proved to be the only hypophysectomized animal reported in this paper in which a small anterior lobe remnant was detectable with the help of a dissection microscope. The effect of progesterone on the other accessory sex organs proved to be the same in these normal and hypophysectomized rats as in the spayed animals described above. The only difference was that since treatment had been continued longer in this series, all changes were much more conspicuous (figs. 9–16).

The oviduct was also examined in this series. While it was but slightly influenced by progesterone in the intact group, the pronounced atrophy produced by hypophysectomy was effectively inhibited by this compound (figs. 17–18).

In conclusion it may be said that our experiments prove that crystalline pure progesterone can exert all typical "corpus luteum hormone actions" on the oviduct, uterus, vagina and mammary gland of the intact rat. Its actions on all accessory organs, except the mammary gland, are independent of the ovary and the hypophysis. Its action on the breast on the other hand, is apparently mediated by the pituitary since it is not observed after hypophysectomy.

The fact that the mammary gland development obtained by progesterone alone in normal or spayed females is rather peculiar in its histological appearance deserves special com-

ment. As we said above—and as our pictures indicate—the parenchyma of the mammary gland is extremely compact and the acinar arrangement of the cells is hardly visible because the lumen is collapsed and practically absent in most cases. This is due to the fact that no trace of milk or colostrum has formed in any of these glands. In this respect the mammogenic effect of progesterone is very different from that of the estrogens which cause marked secretion (often going as far as milk cyst formation, McEuen et al., '36) with very little acinus development. Some secretion into the acini is usually also observed in mammary tissue which is developed under the influence of testosterone (Selye et al., '34 a, b), pregnancy and pseudo-pregnancy (Selye and McKeown, '34 a, b). It is probable that this difference is due to the fact that progesterone stimulates only the mammogenic hormone production without eliciting any prolactin secretion. On the other hand, the observation that in estrin and testosterone-treated animals the newly formed acini are usually distended by a milk-like fluid indicates that in these cases prolactin secretion has also been stimulated to some extent.

In this connection another experiment is of some interest. Six adult spayed female rats received 200 γ of estradiol and six others the same dose of estradiol in combination with 15 mg. of progesterone daily. The mammary glands on the tenth day of treatment were much more developed in the group which received both hormones, but milk was seen in the acini only in the group treated with estrin alone. This observation suggests that progesterone not only fails to stimulate lactation but actually suppresses it, perhaps because it inhibits prolactin formation. It is conceivable that this lactation-inhibiting effect of progesterone is of physiological significance inasmuch as it may be responsible for the absence of any significant amount of secretion during gestation. Conversely the initiation of milk secretion at delivery might be a progesterone withdrawal effect. It is true that estrogens have often been made responsible for these phenomena but our experiments indicate that progesterone has a much more

clear-cut acinus development stimulating and lactation inhibiting action.

Though not quite relevant to the main problem discussed in this communication, it is perhaps worth mentioning that the adrenal glands of the progesterone-treated intact animals weighed only 29 mg. on the average (range: 19–36) while those of the intact controls averaged 44 mg. (range: 33–53). Histological examination showed this atrophy to be almost entirely due to involution of the cortical cells. In this respect the action of progesterone resembles that of the chemically related desoxycorticosterone, although the latter causes much more pronounced cortical atrophy (Selye, '40 b). In view of these findings it appears probable that the failure of Clausen ('40) to obtain a statistically significant decrease in the weight of the adrenals of progesterone-treated rats is merely due to the fact that he worked with male animals and these are much less sensitive to this action of progesterone than females as shown by Selye ('40 b). In the hypophysectomized rats progesterone had no effect on the involution of the adrenal cortex.

SUMMARY AND CONCLUSIONS

Sufficiently large doses of progesterone cause typical progestational transformation of the endometrium, vaginal mucification, enlargement of the preputial glands and full development of the mammary glands in intact or spayed rats.

In hypophysectomized rats, the actions of progesterone on the accessory sex organs are the same as in intact or spayed females except that the mammary glands become entirely irresponsive. It is concluded that progesterone exerts its mammotropic effect by way of the pituitary just as the estrogens and androgens; but unlike the latter, it probably stimulates only the production of mammogenic hormone and causes no prolactin secretion. This is suspected because the mammary glands developed under the influence of progesterone differ from those seen during pregnancy, pseudo-pregnancy and estrogen or androgen treatment in that their acini contain no visible lumen since there is no milk or colostrum secretion.

The oviduct atrophy normally induced by hypophysectomy is prevented by progesterone.

The mammary gland development produced by estrin in spayed female rats is even more pronounced if progesterone is simultaneously given. On the other hand, estrin always causes some milk secretion and this effect is completely prevented by progesterone. Since among all steroid hormones, progesterone is most active in stimulating acinus formation but at the same time inhibits lactation, it is suggested that this hormone rather than estrin may play the most important part in the development of the breasts accompanied by inhibition of secretion which is so characteristic of pregnancy and in the initiation of lactation by hormone withdrawal at parturition.

The adrenals of intact female rats undergo involution of the cortical cells under the influence of large doses of progesterone.

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LITERATURE CITED

ANCEL, P., AND P. BOUIN 1911 Recherches sur les fonctions du corps jaune gestatif. J. Physiol. e. path. gen., p. 31.

ASTWOOD, EDWIN BENNETT, C. F. GESCHICKTER AND E. O. RAUSCH 1937 Development of the mammary gland of the rat. A study of normal experimental and pathologic changes and their endocrine relationships. Amer. J. Anat., vol. 61, p. 373.

ASTWOOD, EDWIN BENNETT, AND G. F. GESCHICKTER 1938 Changes in the mammary gland of the rat produced by various glandular preparations Arch. Surgery, vol. 36, p. 672.

BROUHA, L., AND H. SIMONNET 1929 Recherches expérimentales sur le corps jaune. Ann. de Physiol., vol. 5, p. 567.

CLAUSEN, H. J. 1940 The effect upon the adrenal glands of the administration of large amounts of progesterone to male rats. Amer. Assoc. Anat. Meeting, Louisville.

FEVOLD, H. L., F. L. HISAW AND S. L. LEONARD 1932 Hormones of the corpus luteum. The separation and purification of three active substances. J. Amer. Chem. Soc., vol. 54, p. 254.

FREUD, J. 1938 Genital accessories of female rats. II. Normal and artificial vagina of pregnancy. Acta brevia Neerl., vol. 8, p. 130.

GOMEZ, E. T., AND C. W. TURNER 1938 Further evidence for a mammogenic hormone in the anterior pituitary. Proc. Soc. Exper. Biol. and Med., vol. 37, p. 607.

KAUFMANN, C., AND E. STEINKAMM 1938 Über die Wirkung unphysiologischer Mengen Keim drüsenhormon auf das Genitale weiblicher kastrierter Ratten. Arch. f. Gynäk., Bd. 165, S. 358.

KORENCHEVSKY, VLADIMIR, AND K. HALL 1937 The bisexual and co-operative properties of the sex hormones as shown by the histological investigations of the sex organs of female rats treated with these hormones. J. Path., vol. 45, p. 681.

MCEUEN, C. S., H. SELYE AND J. B. COLLIP 1936 Some effects of prolonged administration of oestrin in rats. Lancet, p. 775.

———— 1937 Role of pituitary in effect of testosterone on the mammary gland. Proc. Soc. Exper. Biol. and Med., vol. 36, p. 213.

NOBLE, R. L. 1939 Direct gynaecogenic and indirect oestrogenic action of testosterone propionate in female rats. J. Endocrinol., vol. 1, p. 184.

REECE, R. P., AND S. L. LEONARD 1939 Further evidence for a mammogenic factor in the rat hypophysis. Proc. Soc. Exper. Biol. and Med., vol. 42, p. 200.

SELYE, HANS 1934 a Nervous and hormonal factors in lactation. Endocrinology, vol. 18, p. 237.

———— 1934 b Further studies on the influence of suckling. Anat. Rec., vol. 60, p. 323.

———— 1935 a Endocrine interrelations during pregnancy. Endocrinology, vol. 19, p. 151.

———— 1935 b Effect of oestrin on ovaries and adrenals. Proc. Soc. Exper. Biol. and Med., vol. 32, p. 1377.

———— 1940 a Activity of progesterone in spayed females not pretreated with estrin. Proc. Soc. Exper. Biol. and Med., vol. 43, p. 343.

———— 1940 b Interactions between various steroid hormones. Canad. Med. Assoc. J., vol. 42, p. 113.

SELYE, HANS, J. S. L. BROWNE AND J. B. COLLIP 1936 Effect of large doses of progesterone in the female rat. Proc. Soc. Exper. Biol. and Med., vol. 34, p. 472.

SELYE, HANS, AND J. B. COLLIP 1936 Fundamental factors in the interpretation of stimuli influencing endocrine glands. Endocrinology, vol. 20, p. 667.

SELYE, HANS, J. B. COLLIP AND D. L. THOMSON 1933 Effect of hypophysectomy upon pregnancy and lactation. Proc. Soc. Exper. Biol. and Med., vol. 30, p. 589.

SELYE, HANS, C. S. MCEUEN AND J. B. COLLIP 1936 Effect of testosterone on the mammary gland. Proc. Soc. Exper. Biol. and Med., vol. 34, p. 201.

SELYE, HANS, AND T. MCKEOWN 1934 a The effect of mechanical stimulation of the nipples on the ovary and the sexual cycle. Surg. Gynec. and Obstet., vol. 59, p. 886.

———— 1935 Studies on the physiology of the maternal placenta in the rat. Proc. Roy. Soc. Lond., vol. 119, p. 1.

TAKEWAKI, KIYOSHI 1938 On the hormonic conditions of pregnancy in the rat. J. of Fac. Sci. Tokyo Imp. Univ., vol. 5, p. 23.

TURNER, C. W., AND A. B. SCHULTZE 1931 A study of the causes of the normal development of the mammary glands of the albino rat. Univ. of Missouri Agricultural Exper. Station Res. Bull. 157, p. 1.

PLATE 1

EXPLANATION OF FIGURES

1 Undeveloped mammary gland of spayed female control. Note that only ducts and fat tissue are visible.

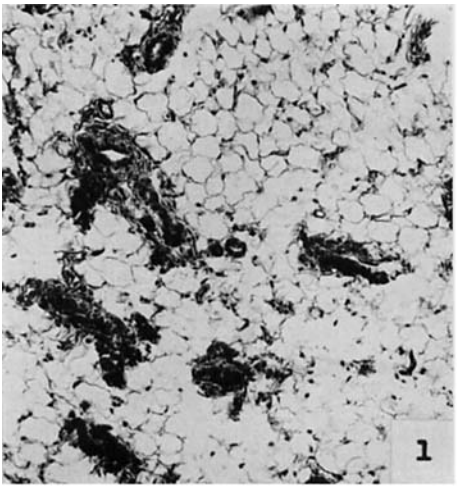
2 Mammay gland of spayed progesterone-treated rat. Note development of acinus tissue and the absence of secretion inside the acini.

3 Uterus of spayed control.

4 Uterus of progesterone-treated spayed rat. Note the well developed and complicated endometrium with numerous crypts and folds. The myometrium is also thickened.

5 Atrophic vaginal epithelium of spayed control.

6 Thickened and mucified vaginal epithelium of progesterone-treated spayed rat. Note beginning cyst formation in epithelium.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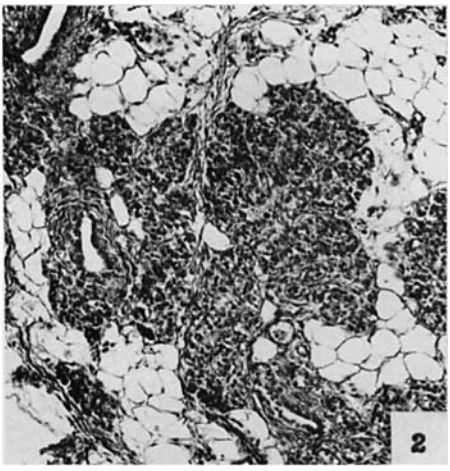
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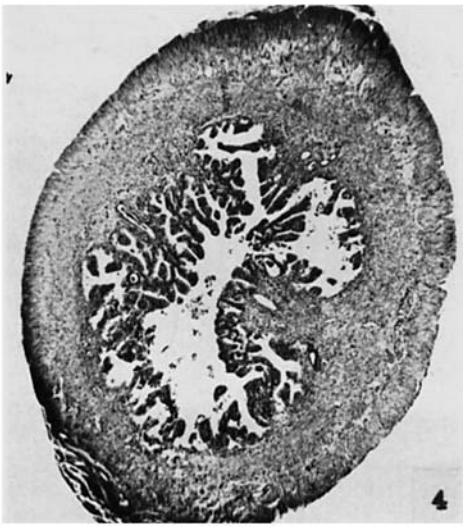
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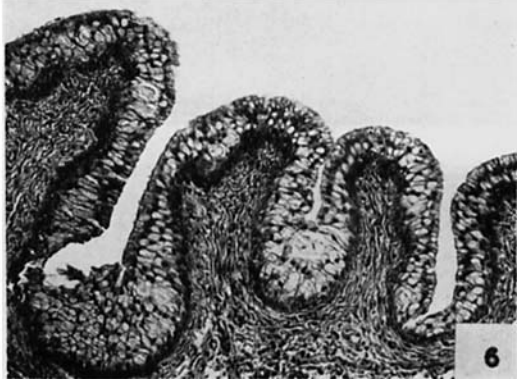
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PLATE 2

EXPLANATION OF FIGURES

7 Mammary gland of progesterone-treated intact rat showing marked acinus development without secretion.

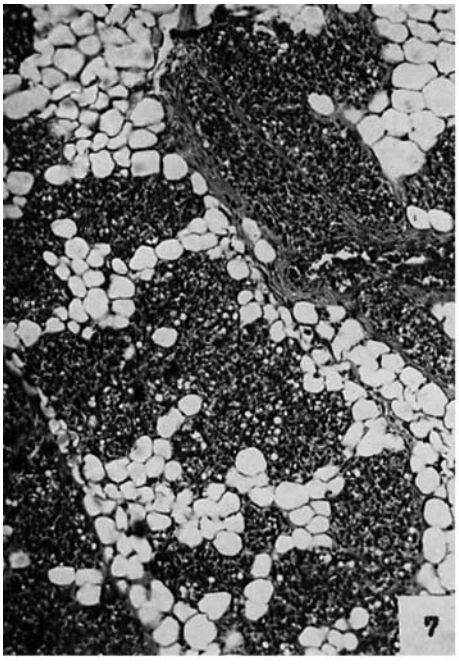
8 Mammary gland of progesterone-treated hypophysectomized rat. The gland is entirely undeveloped and identical in appearance with that of hypophysectomized untreated animals. Only ducts and fat cells are visible.

9 Vagina of intact control rat during diestrus.

10 Vagina of progesterone-treated intact rat displaying marked mucification of the epithelium with cyst formation.

11 Atrophic vaginal epithelium of untreated hypophysectomized rat.

12 Thickened and mucified vaginal epithelium of progesterone treated hypophysectomized rat. Marked intra-epithelial cyst formation.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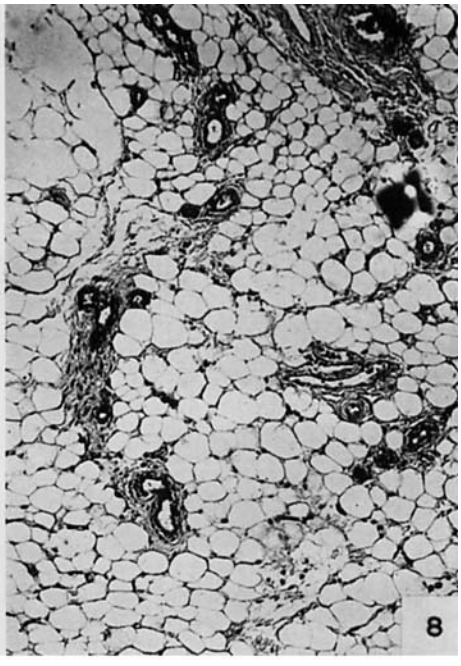
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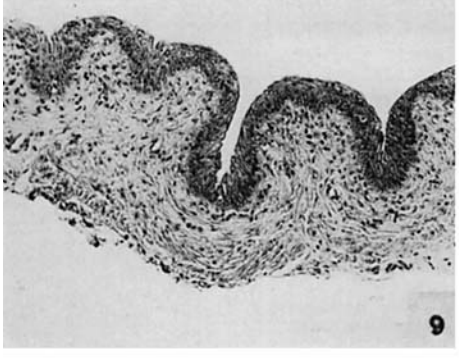
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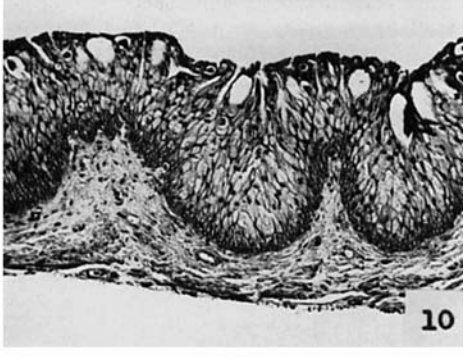
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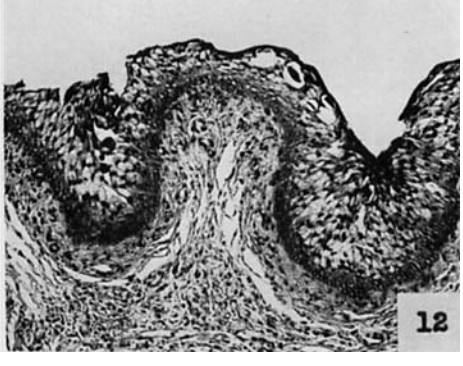
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PLATE 3

EXPLANATION OF FIGURES

13 Uterus of intact rat during diestrus.

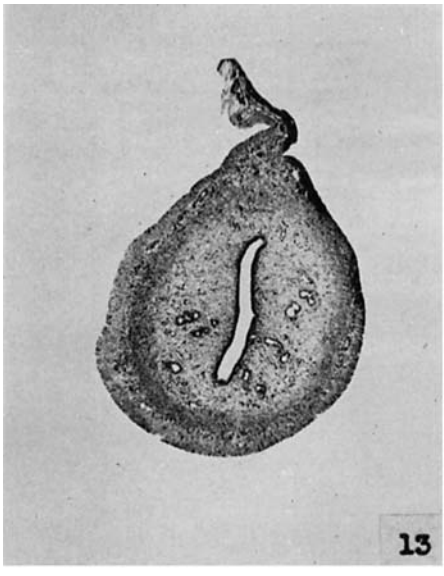
14 Uterus of progesterone-treated rat showing progestational changes similar to those observed in spayed rats treated with this compound (compare with fig. 4).

15 Atrophic uterus of untreated hypophysectomized rat.

16 Progestational uterus in hypophysectomized rat treated with progesterone. The changes are similar to those seen in spayed or intact rats treated with this compound (compare with figs. 4 and 14).

17 Oviduct of hypophysectomized untreated rat. Note atrophy especially of the muscular coat.

18 Oviduct of hypophysectomized progesterone-treated rat. The atrophy was prevented by the treatment.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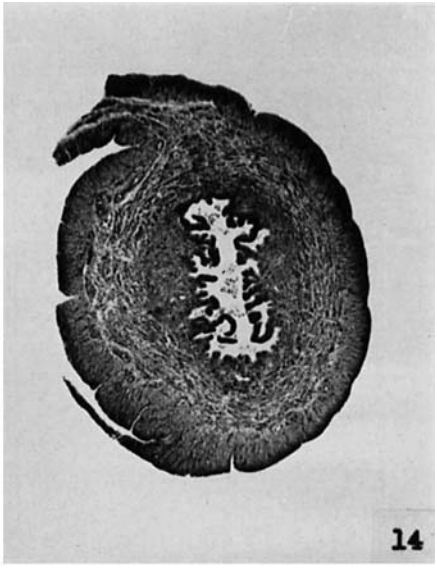
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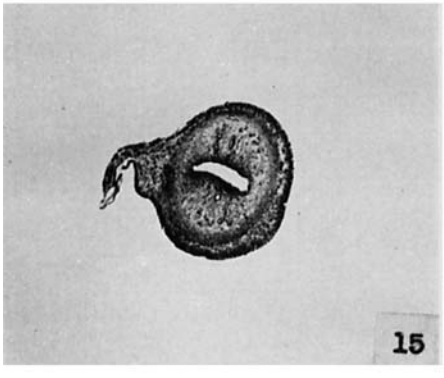
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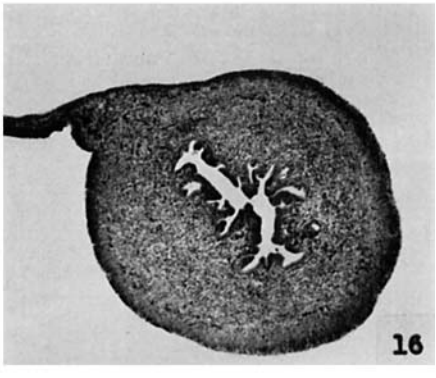
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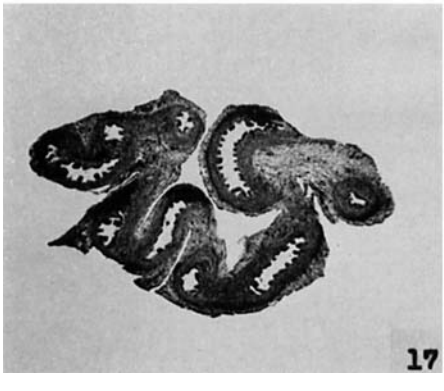
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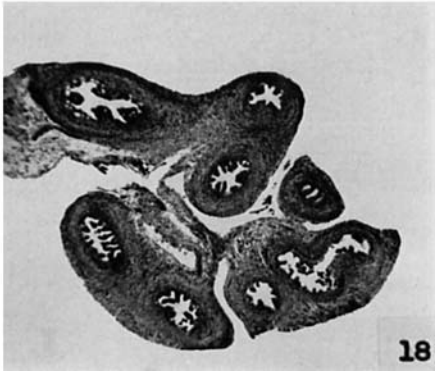
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